IN THE FIGURES:

Please substitute appended Figure 2 and Figure 3 for Figures 2 and 3 as filed. A corrected version showing changes in red ink is also enclosed. Substitute figures are provided merely to correct inadvertent mislabeling of the Figures, and no new matter is presented by these amendments.

IN THE SPECIFICATION:

Please amend the specification by substituting the following paragraphs as indicated below, without prejudice to subsequent renewal of the specification in its original form. These amendments introduce no new matter and are merely provided to correct inadvertent typographical errors, as noted in more detail below.

Page 1, line 17 through 20:

This application claims priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/175,552, filed on January 11, 2000, and 60/181,957, filed on February 10, 2000, the disclosure of each of which is incorporated herein by reference in its entirety for all purposes.

Page 6, line 10 through 12:

~~Figure 6.~~ A series of bar graphs illustrating T cell differentiation in the presence of mDC1 and mDC2: (A) IFN-γ production; (B) IL-5 (open bars) and IL-13 (filled bars) production; (C) ratio of IFN-γ/IL-5 production; and (D) ratio of IFN-γ/IL-13 production.

Page 7, lines 10 through 13:

In contrast with conventional monocyte-derived dendritic cells which strongly favor Th1 differentiation, the unique monocyte-derived dendritic cells of the present invention favor differentiation of Th0/Th2 cells when co-cultured with purified human T cells.

Page 13, lines 22 through 37:

Pathogens and diseased cells, e.g., tumor, necrotic, or apoptotic cells, express a variety of antigens implicated in the cell-mediated immune response against the target cell. It is expected that one of ordinary skill in the art is familiar with the identity of many such antigens.

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T cells recognizing such epitopes are stimulated to proliferate in response to antigen presenting cells, such as dendritic cells, including the dendritic cells of the present invention, which display an antigen on a MHC molecule. Examples of antigens include tumor derived antigens, e.g., prostate specific antigen (PSA), colon cancer antigens (e.g., CEA), breast cancer antigens (e.g., HER-2), leukemia antigens, and melanoma antigens (e.g., MAGE-1, MART-1); antigens to lung, colorectal, brain, pancreatic cancers; antigens to renal cell carcinoma, lung, colorectal, pancreatic B-cell lymphoma, multiple myeloma, prostate carcinomas, sarcomas, and neuroblastomas; viral antigens, e.g., hepatitis B core and surface antigens (HBVc, HBVs), hepatitis A, B or C antigens, Epstein-Barr virus antigens, CMV antigens, human immunodeficiency virus (HIV) antigens, herpes virus antigens, and human papilloma virus (HPV) antigens; bacterial and mycobacterial antigens (e.g., for TB, leprosy, or the like); other pathogen derived antigens, e.g., Malarial antigens from *Plasmodium sp.*; or other cellular antigens, e.g., tyrosinase, trp-1. Many other antigen types are known and available, and can be presented by the DC of the invention.

Page 16, line 25:

Both naturally occurring, wild type and mutant, nucleic acids, as well as engineered or altered nucleic acids are favorably employed in the context of the present invention. One of skill will recognize many ways of generating alterations in a given nucleic acid sequence, such as a known cancer marker which encodes an antigen of interest. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, recursive sequence recombination and diversity generation methods of nucleotides (such as, e.g., DNA shuffling), chemical synthesis of a desired oligonucleotide (e.g., in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. See, e.g., Giliman and Smith (1979) Gene 8:81; Roberts et al. (1987) Nature 328:731; Stemmer (1994) Proc Natl Acad Sci U.S.A. 91:10747; Mullis et al. (1987) U.S. Patent No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) and Sambrook, Ausubel, and Berger (*all supra*).

Page 24, line 33 through page 25, line 6.

Most preferably, cells are isolated and characterized by flow cytometry methods such as fluorescence activated cell sorter (FACS) analysis. A wide variety of flow-cytometry methods are known. For a general overview of fluorescence activated flow cytometry see, for example, Abbas et al. (1991) Cellular and Molecular Immunology, W.B. Saunders Company; and Kuby (1992) Immunology, W.H. Freeman and Company, as well as other references cited above, e.g., Coligan. Fluorescence activated cell scanning and sorting devices are available from e.g., Becton Dickinson, Coulter.

Page 33, lines 25 through 31:

The present invention provides mononuclear cell- or monocyte-derived APC and DC subsets (or subtypes) exhibiting phenotypically and functionally novel properties, features, and characteristics. For clarity and to distinguish these novel dendritic cells from conventional DC, DC of the present invention exhibiting the characteristics, features and properties described herein are termed "mDC2," or dendritic cells (DC) of the present invention. Conventional DC exhibiting commonly known characteristics, features and properties are termed "mDC1" or conventional DC.

Page 34, lines 28 through 32:

The mDC2 of the present invention are further distinguished from mDC1 by their cytokine production profile. MDC2 secrete increased levels of IL-10 compared with mDC1. Additionally, mDC2 produce no IL-12 upon activation with LPS plus IFN-γ or anti-CD40 mAbs, LPS plus IFN-γ, whereas conventional mDC1 cells produce high levels of IL-12 when activated under identical culture conditions.

Page 35, lines 11 through 32:

The mechanisms initiating Th2 cell differentiation have been intensely investigated, because professional APCs, such as DC, are known to produce large quantities of IL-12, the most potent cytokine directing Th1 response. The underlying mechanisms mediating Th2 cytokines IL-4 and IL-13 dominate in certain disease situations, such as allergy resulting in increased IgE production (Punnonen et al. (1993) Proc Natl Acad Sci USA 90:3730; Punnonen et al (1998), in Allergy and Allergic Diseases: The New Mechanisms and Therapeutics (J.

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Denburg ed. Humana Press, Totowa, p.13). IL-4 is well known to efficiently direct Th2 responses, but no IL-4 production has been demonstrated by professional APCs. NK1.1$^+$ T cells, a numerically minor T cell subset, have been shown to produce high levels of IL-4 and are likely to contribute to the initiation of Th2 response (Yoshimoto et al. (1995) Science 270:1845). However, they are not likely to be the only explanation, because APC typically secrete high levels of IL-12. It was recently shown that plasmacytoid cell-derived DC produce low levels of IL-12 and direct Th2 differentiation, whereas monocyte-derived DC produce high levels of IL-12 and skew T cell differentiation towards Th1 (Rissoan et al. (1999) Science 283:1183), indicating that APCs do differ in their capacity to produce cytokines. Importantly, however, two different cell populations were used as the starting material to generate these subsets, and it remained unclear whether one population has the capacity to differentiate DC subsets with different cytokine production profiles and capacities to mediate Th cell differentiation (Rissoan, supra; Bottomly (1999) Science 283:1124). With results described herein and the mDC2 of the present invention demonstrate that PB monoctyes can differentiate into at least two different subsets that differ from each other in cytokine synthesis profile, surface marker expression and capacity to direct Th differentiation.

Page 36, lines 7 through 12:

In contrast to mDC1, mDC2 do not mature into CD83$^+$ DC in the presence of LPS plus IFN-γ, indicating the signaling requirements for maturation between these two DC subsets are not identical. In addition, because mDC1 molecules can act as efficient lipid antigen-presenting molecules (Beckman et al. (1994) Nature 372:691; Sugita et al. (1999) Immunity 11:743), the fact that mDC2 remain CD1a$^-$ upon maturation further supports the belief that the mDC2 subset is phenotypically and functionally distinct from the mDC1 subset.

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Page 37, line 31 through page 38, line 7:

Also included are compositions comprising APC and CD1a$^-$ dendritic cells of the invention. The CD1a$^-$ dendritic cells are capable of presenting an antigen to a T cell. Additionally, in such composition CD1a$^-$ dendritic cells may produce substantially no IL-12 and/or promote differentiation of T cells to a Th0/Th2 subtype. In some such compositions, the

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CD1a- dendritic cells display or present at least one antigen or antigenic fragment thereof. In some such compositions, the at least one antigen or antigenic fragment comprises a protein or peptide differentially expressed on a cell selected from the group consisting of a tumor cell, a bacterially-infected cell, a parasitically-infected cell, a virally-infected cell, and a target cell of an autoimmune response. Such compositions may further comprising a pharmaceutically acceptable carrier, which would be well-known to those of ordinary skill in the art. Certain such compositions may be formulated as a vaccine.

Page 47, lines 16 through 24:

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Dendritic cell vaccines utilizing the monocyte-derived APC or mDC2 of the present invention are useful for cancer immunotherapies, including in therapeutic and prophylactic treatment regimens for the following cancers: prostate cancer; non-Hodgkin's lymphoma; colon cancer; breast cancer; leukemia; melanoma; brain, lung, colorectal, and pancreatic cancers; renal cell carcinoma; and lung, colorectal, pancreatic B-cell lymphoma, multiple myeloma, prostate carcinomas, sarcomas, and neuroblastomas, including those cancers described in Timmerman et al. (1999) Annu. Rev. Med. 50:507-29. The antigens for such cancers are present in Timmerman et al., id. at 523. Such antigens can be presented or displayed on the APC or mDC2 of the invention (using peptide loading, pulsing or transfection methods described above).

Page 53, line 36 through page 54, line 10:

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Relative IL-12 production by DC generated under the culture conditions described above is shown in Figure 1. PB monocytes were cultured in the presence of IL-4 (400 U/ml) and GM-CSF (800 U/ml) in either RPMI (n=15), IMDM (n=4) or Yssel's medium (n=14). In some cultures, IL-6 (100 U/ml) (n=3) or IL-10 (100 U/ml) (n=4) were added at the onset of the cultures, or anti-CD40 mAbs (10 μg/ml) were included on day 5 (n=11) and studied as indicated in the Figure 1. After a culture period of six days, the cells were harvested and activated with LPS (1 ng/ml) plus IFN-γ (10 ng/ml). The supernatants were harvested after culturing for an additional 24 hours, and the levels of IL-12 in the supernatants were measured by ELISA. The results are expressed as mean±SEM.

Page 54, line 14 through 38:

Each of the components of Yssel's medium, namely insulin, transferrin, linoleic acid, oleic acid, and palmitic acid, has been shown to affect the function of lymphoid cells in vitro and/or in vivo (see, e.g., Lernhardt (1990) Biochem. Biophys. Res. Commun. 166:879; Wooten et al. (1993) Cell. Immunol. 152:35; Karsten et al. (1994) J. Cell. Physiol. 161:15; Okamoto et al. (1996) J. Immunol. Meth. 195:7; and Kappel et al. (1998) Scand. J. Immunol. 47:363). To further characterize the culture conditions that favor mDC2 differentiation, we added individual components of Yssel's medium to RPMI, and analyzed IL-12 production and CD1a expression. In addition, because IMDM differs from RPMI in that it contains higher concentrations of glucose, and because glucose has been shown to influence cytokine production by monocytes, with higher glucose concentrations enhancing cytokine production (see, e.g., Morohoshi et al., (1996) "Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro," Diabetes 45:954), we also studied the effect of glucose on differentiation of DC. Addition of glucose at concentrations 4.5 mg/ml and 9.0 mg/ml did not significantly alter or inhibit (n=2) IL-12 production by conventional DC generated in RPMI (compared to DC generated in Yssel's medium), whereas a combination of linoleic acid, oleic acid, and palmitic acid inhibited, but never completely blocked, CD1a expression on mDC1 (data not shown). Nevertheless, under the experimental conditions described herein, no single component of Yssel's medium was able to fully substitute the effect of the complete medium in inducing altered cytokine production in differentiated DC cells (i.e., differentiation of mDC2) (data not shown). Moreover, if the monocyte cultures were initiated with RPMI, and Yssel's medium was added after 24 hours after the onset of the cultures, the cells differentiated into conventional mDC1 producing high levels of IL-12 upon activation (data not shown), demonstrating that DC differentiation into subsets with different cytokine production profiles is dependent on a delicate balance of growth factors that are present during the initial stages of DC differentiation.

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Page 55, lines 18 through 27:

No significant difference in the mean fluorescence intensity (MFI) of these antigens was observed irrespective of whether the cells were differentiated in the presence of

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RPMI or Yssel's medium. In addition, no differences in the expression levels of CD13, CD23, CD32, CD33, CD54, and MHC class I molecules between these DC populations were observed, and both subsets (subtypes) also expressed CD47 (data not shown). Furthermore, the DC differentiated either in the presence of Yssel's medium or RPMI strongly downregulated expression of CD14 (as an indication of differentiation into DC) (Fig. 2), demonstrating a phenotype of conventional DC. As a control, monocytes differentiated in the presence of M-CSF in either medium differentiated into macrophages expressing high levels of CD14 with macroscopic appearance of macrophages (data not shown).

Page 57, line 25 to page 58, line 6:

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The cytokine production profiles of mature mDC1 and mDC2 were essentially the same as those of the corresponding CD83- population subsets. Regarding IL-12 production, supernatants of mature mDC1 contained 2897±937 picogram/milliliter (pg/ml) IL-12 (mean±SEM), whereas those of mDC2 derived from the same donors contained 125±93 pg/ml IL-12 (n=10). Specifically, in 8 out of 10 experiments, IL-12 production from mature mDC2 was undetectable in ELISA assays in which IL-12 sensitivity is 5 pg/ml. The average of mature mDC2 IL-12 production of 10 experiments was 125±93 pg/ml IL-12 (n=10). The term "substantially lacks IL-12 production," "substantially lacking in production of IL-12," "substantially decreased production of IL-12," or "produces substantially no IL-12" in reference to mature mDC2 IL-12 production refers to a substantial decrease or substantial lack in mature mDC2 IL-12 production relative to the mature mDC1 IL-12 production, and typically refers to a mature mDC2 IL-12 production ranging from at least about 50% to about 100% less, at least about 60% to about 100% less, at least about 70% to about 100% less, at least about 80% to about 100% less, at least about 90% to about 100% less, at least about 95% to about 100% less, at least about 97% to about 100% less, or at least about 99% to about 100% less, than mature mDC1 IL-12 production.

Page 58, lines 7 through 19:

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Regarding IL-10, IL-10 production was undetectable in cultures of mature mDC1 (using the ELISA assays in which IL-10 sensitivity is 5 pg/ml), whereas 215±23 pg/ml